Postmenopausal hormone replacement therapy modifies skeletal muscle composition and function: a study with monozygotic twin pairs

Paula H. A. Ronkainen,1,2 Vuokko Kovanen,1,2 Markku Alén,1,3 Eija Pöllänen,1,2 Eeva-Maija Palonen,1,2 Carina Ankarberg-Lindgren,4 Esa Hämäläinen,5 Ursula Turpeinen,5 Urho M. Kujala,1 Jukka Puolakka,6 Jaakko Kaprio,7,8 and Sarianna Sipilä1,2

1Department of Health Sciences and 2Finnish Centre for Interdisciplinary Gerontology, University of Jyväskylä, Jyväskylä and 3Department of Medical Rehabilitation, Oulu University Hospital and Institute of Health Sciences, University of Oulu, Oulu, Finland; 4Department of Pediatrics, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden; and 5HUCLAB, Helsinki University Central Hospital, Helsinki, 6Central Finland Central Hospital, Jyväskylä, and 7Department of Public Health and Institute for Molecular Medicine, University of Helsinki and 8Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland

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Postmenopausal hormone replacement therapy modifies skeletal muscle composition and function: a study with monozygotic twin pairs. J Appl Physiol 107: 25–33, 2009. First published February 26, 2009; doi:10.1152/japplphysiol.91518.2008.—We investigated whether long-term hormone replacement therapy (HRT) is associated with mobility and lower limb muscle performance and composition in postmenopausal women. Fifteen 54- to 62-yr-old monozygotic female twin pairs discordant for HRT were recruited from the Finnish Twin Cohort. Habitual (HWS) and maximal (MWS) walking speeds over 10 m, thigh muscle composition, lower body muscle power assessed as vertical jumping height, and maximal isometric hand grip and knee extension strengths were measured. Intrapair differences (IPD%) with 95% confidence intervals (CI) were calculated. The mean duration of HRT use was 6.9 ± 4.1 yr. MWS was on average 7% (0.9 to 13.1%, P = 0.019) and muscle power 16% (−0.8 to 32.8%, P = 0.023) greater in HRT users than in their cotwins. Thigh muscle cross-sectional area tended to be larger (IPD% 0.065), relative muscle area greater (IPD% 0.047), and relative fat area smaller (IPD% 0.047) in HRT users than in their sisters. There were no significant differences in maximal isometric strengths or HWS between users and nonusers. Subgroup analyses revealed that estrogen-containing therapies (11 pairs) significantly decreased total body and thigh fat content, whereas tibolone (4 pairs) tended to increase muscle cross-sectional area. This study showed that long-term HRT was associated with better mobility, greater muscle power, and favorable body and muscle composition among 54- to 62-yr-old women. The results indicate that HRT is a potential agent in preventing muscle weakness and mobility limitation in older women.

aging; menopause; sex hormones; muscle strength; muscle power

LOW MUSCLE STRENGTH among middle-aged and older people is predictive of adverse health events (45) as well as incident mobility limitation and disability (44). Among the major reasons for muscle weakness and consequent mobility limitation are the inexorable age-induced changes in muscle composition such as loss of muscle tissue (59). Cross-sectional computed tomography (CT) has shown that aging is accompanied not only by loss of muscle mass but also by fat infiltration within the muscle and between the separate muscle groups. Moreover, it has been suggested that loss of muscle mass contributes to fat gain, which again exerts adverse effects on muscle mass (31).

Aging is characterized by a decline in endocrine activity. In women, the most dramatic hormonal change is observed during the peri- and postmenopausal periods, when the secretion of ovarian hormones, namely, estradiol and progesterone, is drastically reduced. By the mid-sixth decade of life, all women have gone through the menopause, which means that on average women spend one-third of their lives in a state of hypogonadism. Interestingly, some cross-sectional studies in older women have shown that higher serum estradiol concentration is associated with greater muscle mass (21, 54) and muscle strength (49, 54). In addition, a few studies have indicated that an accelerated decline in muscle strength occurs right at the time of menopause (24, 38, 47). These data imply that a low level of female sex steroids may represent a potential mechanism for aging-related muscle weakness and unfavorable changes in muscle and body composition, thus increasing the risk for mobility limitation and disability.

In previous randomized controlled trials (RCTs), administration of postmenopausal hormone replacement therapy (HRT) for 6 mo to 1 yr has been documented to improve mobility and increase muscle strength among relatively young postmenopausal women (50, 53, 55), whereas among older women similar effects with treatment lasting 6 mo to 3 yr were not found (27, 46). To the best of our knowledge, only two RCTs have investigated the effects of HRT on muscle cross-sectional area (CSA) and muscle composition in postmenopausal women. First, an open trial by Skelton and colleagues (53) showed no change in the CSA of the muscle adductor pollicis after 1 yr of cyclical estrogen and norgestrel treatment in women with a mean age of 61 yr despite a significant gain in strength of the same muscle. Second, a double-blind RCT among early postmenopausal women showed a significant 6% mean increase in the CSA of the knee extensor muscle after continuous combined estradiol and progesterin treatment compared with control subjects (50). In the same study, thigh muscle density assessed by CT increased after HRT (55), suggesting decreased intramuscular lipid content (14). In addition, the relative proportion of fat within the knee extensor...
muscle compartment remained unchanged in the women on HRT, whereas it increased in the control group (50).

During the last six years the risks and benefits concerning long-term use of postmenopausal HRT have been evaluated by large clinical trials. However, there is little knowledge on the possible preventive effects of long-term HRT on mobility limitation and disability. The reason for this gap in research area is that the clinically relevant aging-induced features, such as the loss of muscle mass, muscle weakness, and mobility limitation, develop over several years. An effective strategy for studying the long-term effects of HRT is to utilize a cotwin control design, in which exposed monozygotic (MZ) twins are compared with their unexposed cotwins. This design presents an incomparable opportunity to examine “clonal controls” and to study the associations of interest independently of individual genetic makeup and shared past experiences from childhood onward. Because MZ twins are genetically identical at the sequence level, any difference observed between cotwins must be founded on acquired factors. The purpose of this study was to investigate whether long-term HRT results in clinically significant alterations in mobility and skeletal muscle mass, composition, and function in 54- to 62-yr-old postmenopausal women.

**MATERIALS AND METHODS**

**Study Design**

This study is part of a larger research project, “Sarcopenia—Skeletal Muscle Adaptation to Postmenopausal Hypogonadism and Effects of Hormone Replacement Therapy and Physical Activity in Older Women: a Genetic and Molecular Biological Study on Estrogen-related Pathways” (SAWES), which was set up to investigate the molecular events that participate in the regulation of muscle mass and function after menopause. The participants were recruited from the Finnish Twin Cohort (25, 26), which includes all same-sex twin pairs born in Finland before 1958 with both cotwins alive in 1967 (n = 13,888 pairs). Health- and lifestyle-related factors were assessed in the Finnish Twin Cohort by specific questionnaires mailed to the participants in 1975, 1981, and 1990 (25). An invitation to the present study and were further invited to the laboratory examinations. The zygosity of the twins participating in the laboratory measurements was verified at the Paternity Testing Laboratory, National Public Health Institute with DNA extracted from a venous blood sample with a battery of 10 highly polymorphic gene markers. Fifteen of the sixteen pairs participating in the measurements were confirmed to be MZ pairs, whereas one twin pair turned out to be dizygotic (DZ) and was excluded from the present analyses.

Of the HRT users five women used estradiol-only preparations (amount of estrogenic agent in preparation was 1–2 mg), whereas six were taking a combined treatment including estrogenic (1–2 mg) and progestogenic compounds. Four women used tibolone (2.5 mg), which represents an analog of progestin and is metabolized in the intestine and the liver into metabolites that have both estrogenic and progestogenic/androgenic effects on target tissues (28). Thirteen women were taking preparations as pills, one used a hormonal patch, and one used a gel preparation. Of the nonusers, one cotwin had tested positive for progesterone (DZ) and was excluded from the present analyses.

Each participant took part in the laboratory measurements during two consecutive days. CT scans were conducted at the local hospital on the first measurement day, while all the other laboratory measurements were carried out on the second day. Both members of a twin pair participated in all the measurements on the same days. All the

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**Fig. 1.** Flow chart of the study. HRT, hormone replacement therapy.
laboratory measurements and data analysis were carried out by personnel blind to HRT status.

The Ethics Committee of the Central Finland Health Care District approved the study, and it was conducted according to the guidelines laid down by the World Medical Association in the Declaration of Helsinki (2000). Written informed consent was provided by the participants before the measurements.

**Blood Sampling, Medical Examination, and Health Status**

Fasting blood samples were taken from the antecubital vein in a supine position between 0700 and 0900. Sera stored at −70°C after sampling were used for the hormone measurements as described below. During a medical examination a physician assessed the participant’s general health status and gynecologic history and confirmed the presence of possible chronic diseases. The examination included a detailed review of the use of medication, including the history of HRT. Moreover, the possible presence of contraindications for participation in the study was confirmed. Data on current and past smoking were collected with a standard questionnaire.

**Hormone Measurements**

Serum follicle-stimulating hormone (FSH) and sex hormone-binding globulin (SHBG) concentrations were measured with solid-phase, chemiluminescent immunometric assays (Immulite 1000, Diagnostic Products, Los Angeles, CA). The interassay coefficients of variation (CVs) were 5.5% for concentrations of 38.5 IU/l (FSH) and 8.4% for 32.4 nmol/l (SHBG). The limit of quantification (LOQ) was 0.1 IU/l for FSH and 0.2 nmol/l for SHBG. Serum 17β-estradiol (E2) levels were determined in duplicate by extraction RIA as previously described (3). Extraction RIA has been validated especially for measuring low serum E2 concentrations. LOQ was 4 pmol/l, while interassay CV was 19% at 6 pmol/l and <14% for concentrations of 12 pmol/l and above. Serum testosterone (T) was measured as previously described (57). LOQ was 70 pmol/l and interassay CV was 5.2% for the concentration 4.7 nmol/l. E2 and T levels were utilized together with SHBG in calculating the respective free hormone levels according to previously presented methods (6, 58). Estrone (E1) was determined as a dansyl derivative by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with an API 4000 mass spectrometer as previously described (35). Interassay CV was 7.8% for the concentration of 200 pmol/l, while LOQ was 10 pmol/l.

**Body Anthropometry and Composition**

Body anthropometry was measured between 0700 and 1000 after overnight fasting with the participant wearing only undergarments. Body weight was measured in kilograms with a beam scale and height in centimeters with a stadiometer while the participant was standing in stocking feet. Waist circumference was measured midway between the greater trochanters, both to the nearest half centimeter. Percentage body fat and lean body mass (LBM) were measured with a multifrequency bioelectrical impedance analyzer, which takes readings from the body with an eight-point tactile electrode method [InBody (720), Biospace, Seoul, Korea]. In our laboratory the CV for two consecutive measurements of percent body fat is 0.6% (61). Self-report data on weight and height from prior questionnaires completed by the twins in 1975, 1981, and 1990 of the twin cohort were also accessed, and body mass index (BMI) was computed for each time point.

**Mobility**

Mobility was assessed with habitual (HWS) and maximal (MWS) walking speed for 10 m in a laboratory corridor. Five meters were allowed for acceleration, and the time taken to walk 10 m was measured with photocells. In the HWS test the participants were instructed to “walk at your normal speed, as if you were going to a supermarket without a need to hurry,” whereas in the MWS test the participants were advised to “walk as fast as possible, without compromising your safety.” Two trials were conducted for each test, and the faster performance was documented as the result. The participants wore their own walking shoes or sneakers. For safety purposes, the examiner walked behind the participant during the tests. Walking speed is highly predictive of mobility limitation and disabilities related to activities of daily living (17). In our laboratory the CV for MWS has been 5% (37).

**Thigh Muscle mass and composition.** CT scans (Siemens Somatom Emotion scanner, Siemens, Erlangen, Germany) were obtained from the midpoints between the greater trochanter and the lateral joint line of the knee. The scans were analyzed with software developed at the University of Jyväskylä for cross-sectional CT image analysis (Geanix 2.1, Commit, Espoo, Finland), which separates fat and lean tissue on the basis of the given radiological density limits. Total thigh muscle and fat (includes subcutaneous fat and fat infiltrated into the muscle compartment) CSAs were analyzed, and the relative proportions of muscle and fat within the whole thigh CSA were calculated. Fat area within the muscle compartment (infiltrated fat) was determined by manually outlining the muscle compartment by drawing a line along the fascial plane to exclude subcutaneous fat. CSAs and relative proportions of muscle and fat as well as muscle attenuation were analyzed. Skeletal muscle attenuation was defined as the mean attenuation coefficient in Hounsfield units (HU). In our previous studies, the CV between two consecutive measurements was 1–2% for muscle CSA (50) and <1% for muscle HU (55).

**Isometric muscle strength and lower body muscle power.** Maximal isometric knee extension strength was measured on both legs in a sitting position with an adjustable dynamometer chair at a knee angle of 60° from full extension (Good Extension, Metituri, Palokka, Finland). In addition, hand grip strength was measured on the dominant side by fixing the arm to the armrest of the chair with the elbow flexed at an angle of 90° (41, 42). The participants were instructed to lift their leg/squeeze the handle with as much force as possible and make the best effort they could each time. The contraction was maintained for 2–3 s. After two or three practice trials, the measurement was performed at least three times until no further improvement occurred. Depending on the participant, this stage was reached after three to six maximal efforts. Lower body muscle power, i.e., the ability of the neuromuscular system to produce the greatest possible force as fast as possible, was assessed as the height that the participant was able to elevate her body’s center of gravity during a vertical jump (vertical jumping height) on a contact mat. Flight time was measured, and height was calculated as follows: vertical jumping height (cm) = \((g \times t^2) + 8 \times 100\) (7). Three maximal efforts were conducted. In all the measurements, the best performance with the highest value was accepted as the participant’s score. In our laboratory the CVs between two consecutive measurements earlier had been 6% for knee extension strength and hand grip strength and 5% for vertical jumping height (50).

**Physical Activity**

Physical activity was assessed with the scale of Grimby (16), with slight modifications. The participants were categorized on the basis of their self-reported physical activity into groups labeled sedentary (no other activities, but at the most light walking ≤2 times/wk), moderately active (walking or other light exercise at least 3 times/wk, but no other more intensive activities), and active (moderate or vigorous exercise at least 3 times/wk). Physical activity during leisure was also assessed in questionnaires administered in 1975, 1981, and 1990 (30).
Energy Intake

Daily energy intake was assessed by a 5-day food record encompassing three weekdays and two weekend days. The records were analyzed with Micro-Nutrica software (version 2.5, Social Insurance Institution of Finland).

Statistical Analyses

Owing to the relatively small number of observations, the statistical significance of the differences between the means in HRT users and nonusers was tested with Wilcoxon’s signed rank test. Data are shown as means and SDs unless otherwise stated. Intrapair differences are expressed as percentages (IPD%) and calculated as follows: (HRT user − nonuser) ÷ (nonuser) × 100. In addition, the 95% confidence interval (CI) was calculated for the IPD%. Data are reported for the whole group (“HRT users”, n = 15 pairs) and separately for two subgroups: “E users,” consisting of the pairs in which the HRT cotwin used either an estradiol-only or a combined estradiol plus progesterone preparation (n = 11 pairs) and “tibolone users,” who used a preparation containing tibolone as an effective agent (n = 4 pairs). With the present sample size (15 discordant pairs), the statistical power of detecting a significant (P < 0.05) difference between the sisters was between 0.43 and 0.66 for thigh muscle area, thigh relative muscle area and fat area, vertical jumping height, and maximal walking speed and <0.43 for the other variables. The level of significance was set at P ≤ 0.05. Data analyses were carried out with SPSS (version 14.0, SPSS, Chicago, IL).

RESULTS

Participants’ Characteristics and Hormonal Status

The mean age (±SD) of the participants was 57.2 ± 1.8 yr (range 54–62 yr). Table 1 presents the participants’ health status and living habits according to the use of HRT. According to the questionnaires administered in 1975, 1981, and 1990, there were no differences in physical activity, smoking behavior, or alcohol use between HRT users and their nonusing cotwins before the use of HRT. There were no differences in daily energy intake between HRT users and nonusers (1,535 ± 319 vs. 1,632 ± 261 kcal, P = 0.39). Moreover, the relative (%) amount of energy obtained from proteins, fat, or carbohydrates in the total daily energy intake was similar between the sisters (P = 0.61–0.87). The observed differences between HRT users and nonusers in serum hormone concentrations were as expected (Table 2). Concentrations of E2 and E1 in HRT users were on average five times higher than in nonusers. A similar trend was observed for free E2 as well. No difference was observed between the sisters in the levels of SHBG or in total or free T. Subgroup analyses of total and free E2 and E1 showed that the differences observed between users and nonusers were primarily due to differences between E users and their cotwins. Furthermore, SHBG levels were on average 62% (P = 0.010) higher in E users than in their cotwins, whereas a trend toward 54% (P > 0.05) lower SHBG levels was observed in tibolone users than in their sisters. It should be noted that the assays utilized do not measure serum tibolone.

Anthropometry and Total Body and Muscle Composition

HRT users were on average 0.8 cm taller than nonusers (IPD% = 0.5%, P = 0.025; Table 3). No statistically significant differences were observed in weight, BMI, waist or hip circumference, LBM, or body fat percentage between the sisters. In a subgroup analysis, however, the E users had lower percent body fat (P = 0.026) and tended to have lower BMI (P > 0.05) compared with their cotwins with no history of HRT. According to previous self-reports, the sister currently using HRT was already taller than her cotwin in 1975, 1981, and 1990, although not statistically significantly (P = 0.054–0.399). Moreover, the sisters within a pair did not differ in BMI before the use of HRT.

A trend toward 6% greater total thigh muscle CSA was observed among HRT users compared with nonusers (P > 0.05, Table 4). A subgroup analysis showed a trend toward 15% larger mean total thigh muscle CSA (P > 0.05) for the tibolone users compared with their cotwins, while a 3% non-significant difference within the E-using twin pairs was found.

No significant differences were observed in total thigh fat and subcutaneous fat between the sisters. However, the subgroup analysis revealed that total thigh fat CSA, including subcutaneous fat and fat infiltrated into the muscle compartment, was on average 12% (P = 0.021) lower in E users than in their cotwins. Moreover, E users had less subcutaneous fat (IPD% = 10%, P = 0.037) and tended to have a lower amount of infiltrated fat (IPD% = 10%, P > 0.05) than their sisters.

Muscle CSA in relation to total soft tissue CSA of the thigh (relative muscle area of thigh) was on average 8% (P = 0.047) larger among HRT users than nonusers. When subcutaneous fat was excluded from the analysis and muscle CSA was analyzed in relation to muscle compartment CSA (relative muscle area of muscle compartment), HRT users showed a trend toward an average of 1% (P > 0.05) larger relative muscle area within the thigh muscle compartment compared with their sisters with no history of HRT.

The larger relative muscle areas among HRT users appeared to be due to the higher proportion of muscle in E users [relative muscle area of the total thigh was 11% (P = 0.013) and relative muscle area of the muscle compartment 2% (P = 0.033) larger in E users than in their sisters]. This was probably due to the smaller amount of subcutaneous and infiltrated fat in the thigh in combination with the slightly larger muscles among E users compared with their cotwins.

Mobility

The MWS of HRT users was 7% greater compared with that of nonusers (2.2 ± 0.3 vs. 2.0 ± 0.2 m/s, P = 0.019; Fig. 2). The same trend indicating that HRT users were faster walkers than nonusers was also observed after division into subgroups (E users: 2.1 ± 0.3 vs. 2.0 ± 0.2 m/s, P > 0.05; tibolone users:...
2.3 ± 0.3 vs. 2.1 ± 0.1 m/s, P > 0.05). HWS did not differ between HRT users and nonusers.

**Muscle Strength and Power**

Lower body muscle power assessed as vertical jumping height was on average 16% higher in greater users compared with their cotwins (14.8 ± 3.7 vs. 13.2 ± 3.7 cm, P = 0.023; Fig. 2). This difference was clearly due to better muscle power among the E users, who were able to elevate their body on average 21% higher than their twin sisters with no history of HRT (15.1 ± 4.2 vs. 13.0 ± 4.3 cm, P = 0.016). No intrapair differences were observed within the tibolone group (users vs. nonusers: 14.1 ± 3.2 vs. 13.9 ± 1.7 cm, P > 0.05, IPD% = 1.8%). There were no significant differences in maximal isometric strength between HRT users and nonusers.

**DISCUSSION**

In seeking to elucidate the effects of postmenopausal HRT on muscle properties, we investigated the association of long-term HRT with mobility and lower limb muscle characteristics in a rare sample of postmenopausal monozygotic twin pairs. We found, first, that HRT was associated with better mobility and greater muscle power among the 54- to 62-yr-old women. In addition, the sisters using HRT had a lower relative proportion of body fat. These results suggest that HRT may have a direct effect on muscle mass and strength, and may contribute to the overall positive impact of HRT on physical performance observed in this sample of postmenopausal women.
of fat within the thigh and tended to have larger muscles than their genetically identical sisters with no HRT history. Subgroup analysis on muscle composition revealed that estrogen-containing therapies primarily influenced fat tissue and consequently also the relative proportion of muscle.

The greater MWS found in HRT users compared with their sisters irrespective of the HRT preparation used is a finding of notable importance. Walking speed is widely used as a key indicator of mobility limitation and disability in old age (17, 43). Difficulty in walking is associated with adverse health events (19) and also increases dependence on social and health care services (62). Fast and safe walking is a multifactorial task requiring sufficient neuromuscular performance as well as postural control (43). The mechanism underlying greater walking speed in cotwins using HRT compared with their sisters may include larger thigh muscles, greater lower body muscle power, and lower percentage body fat. Earlier studies have documented that high body fat mass and BMI are significantly associated with mobility limitation (60, 65). In particular, among older persons who are overweight and have low muscle strength or muscle mass (4, 65) the prevalence of and the risk for physical disability is substantial. Supporting our finding, a significant association between estrogen-containing therapy and walking speed (48, 54).

Our participants with estrogen-containing therapy had >20% greater muscle power compared with their sisters. These results are supported by our previous study (50) among early postmenopausal women, in which 1-yr administration of combined estradiol and progesterone treatment improved jump-

### Table 4. Muscle composition of MZ twin pairs discordant for long-term use of HRT

<table>
<thead>
<tr>
<th>Variable</th>
<th>HRT Users</th>
<th>Nonusers</th>
<th>Intrapair Difference (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total thigh muscle cross section</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thigh muscle area, mm²</td>
<td>9,626.7 ± 1,171.6</td>
<td>9,153.3 ± 1,301.6</td>
<td>6.0 (−0.07 to 12.1)</td>
<td>0.065</td>
</tr>
<tr>
<td>E users</td>
<td>9,627.3 ± 1,169.0</td>
<td>9,410.9 ± 1,157.7</td>
<td>2.8 (−4.2 to 9.9)</td>
<td>0.51</td>
</tr>
<tr>
<td>Tibolone users</td>
<td>9,625.0 ± 1,360.4</td>
<td>8,445.0 ± 1,589.3</td>
<td>14.8 (3.1 to 26.5)</td>
<td>0.068</td>
</tr>
<tr>
<td>Relative muscle area, %</td>
<td>53.5 ± 9.5</td>
<td>50.4 ± 11.2</td>
<td>3.1 (1.3 to 11.1)</td>
<td>0.013</td>
</tr>
<tr>
<td>E users</td>
<td>54.2 ± 10.0</td>
<td>49.8 ± 12.7</td>
<td>4.4 (1.6 to 11.2)</td>
<td>0.047</td>
</tr>
<tr>
<td>Tibolone users</td>
<td>51.7 ± 9.1</td>
<td>52.2 ± 6.9</td>
<td>0.5 (−0.9 to 2.0)</td>
<td>0.72</td>
</tr>
<tr>
<td>Muscle attenuation, Hu</td>
<td>53.4 ± 4.4</td>
<td>53.3 ± 5.3</td>
<td>0.9 (−5.0 to 6.8)</td>
<td>0.26</td>
</tr>
<tr>
<td>E users</td>
<td>53.3 ± 4.7</td>
<td>53.4 ± 6.0</td>
<td>0.6 (−7.8 to 9.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>Tibolone users</td>
<td>53.6 ± 3.9</td>
<td>52.8 ± 3.2</td>
<td>1.6 (−1.0 to 4.1)</td>
<td>0.14</td>
</tr>
<tr>
<td>Thigh fat area, mm²</td>
<td>8,734.0 ± 3,038.4</td>
<td>9,992.0 ± 5,458.1</td>
<td>−3.0 (−20.5 to 14.5)</td>
<td>0.14</td>
</tr>
<tr>
<td>E users</td>
<td>8,598.2 ± 3,399.6</td>
<td>10,786.4 ± 6,170.8</td>
<td>−11.9 (−28.5 to 4.7)</td>
<td>0.021</td>
</tr>
<tr>
<td>Tibolone users</td>
<td>9,107.5 ± 2,074.9</td>
<td>7,807.5 ± 1,848.7</td>
<td>21.5 (−41.1 to 84.1)</td>
<td>0.27</td>
</tr>
<tr>
<td>Relative fat area, %</td>
<td>46.5 ± 9.5</td>
<td>49.6 ± 11.2</td>
<td>−5.0 (−11.3 to 1.2)</td>
<td>0.047</td>
</tr>
<tr>
<td>E users</td>
<td>45.8 ± 10.0</td>
<td>50.2 ± 12.7</td>
<td>−7.4 (−13.3 to 1.5)</td>
<td>0.013</td>
</tr>
<tr>
<td>Tibolone users</td>
<td>48.3 ± 9.1</td>
<td>47.8 ± 6.9</td>
<td>1.4 (−24.4 to 27.2)</td>
<td>0.72</td>
</tr>
<tr>
<td>Subcutaneous fat area, mm²</td>
<td>7,391.3 ± 2,645.0</td>
<td>8,542.9 ± 4,979.4</td>
<td>−1.3 (−20.8 to 18.2)</td>
<td>0.20</td>
</tr>
<tr>
<td>E users</td>
<td>7,340.9 ± 3,000.6</td>
<td>9,294.0 ± 5,605.7</td>
<td>−9.9 (−31.2 to 11.3)</td>
<td>0.037</td>
</tr>
<tr>
<td>Tibolone users</td>
<td>7,530.0 ± 1,613.0</td>
<td>6,477.5 ± 1,790.6</td>
<td>22.5 (−37.3 to 82.2)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD for 15 total pairs, 11 pairs in which the HRT user was on estradiol only or combined HRT (E users), and 4 pairs in which the HRT user was on tibolone (tibolone users). HU, Hounsfield unit.
ing height on average by 7% compared with the 5% decrease in the placebo group. Muscle power is the product of force and speed of contraction, and thus represents the ability of the neuromuscular system to produce the greatest possible force as fast as possible. Earlier studies have shown that muscle power is more sensitive to the aging process and that it may be more important in mobility than muscle force alone (3, 52). To date, it has been accepted that the fluctuation in circulating estradiol levels is not immediately translated into changes in muscles themselves, as rapid experimental changes in estradiol in postmenopausal (53) or hypoestrogenic (15) women have been reported not to produce acute changes in muscle function. Given the reported neuroprotective effects of estradiol (9, 13), the difference between the sisters in dynamic muscle power, but not in strength, may rather be due, at least in part, to the possible effects of HRT on peripheral neurons.

In our participants HRT was not associated with isometric muscle strength. Recent reports disagree over whether the use of HRT is associated with muscle strength or not. It has been documented in a number of previous studies that 6 mo to 1 yr of combined HRT improves muscle isometric muscle strength (50, 53, 64) or preserves strength, at least to some extent (36, 38). Studies reporting no association have also been published (e.g., Ref. 46). The discrepancy observed between the results of different studies may be due to several methodological issues such as differences in study design, age of participants, time since menopause, test conditions or equipment used, muscles studied, duration of HRT, and the amount and type of effective agent in the preparations.

The present study suggests that HRT tends to be associated with larger thigh muscles. A trend toward IPD% of 15% was observed between tibolone users and their sisters, whereas no difference between E users and their sisters was found. Earlier case-control (54) and RCT (50) studies showed a 3–6% significant difference in thigh muscle mass between women using estrogen-containing HRT and nonusers. In the latter study, the treatment included continuous combined estradiol (2 mg, high dose) and norethisterone acetate (1 mg). On the other hand, the RCT by Kenny et al. (27) showed no effect of 3-yr ultra-low-dose estrogen (0.25 mg) therapy on appendicular muscle mass in women over 65 yr. In our case-control study, most of the sisters were using products containing 1 mg of the estrogenic effective agent. The discrepancy in muscle CSA between the earlier RCTs and the present study may be due to the differences in the preparations and doses used in these studies, and to the age of the participants. We were unable to identify any previous reports specifically on the effects of tibolone on muscle mass. In our study, the small sample size within the tibolone group weakens the conclusions that can be drawn from the results. Nonetheless, we found a gradient toward greater muscle size and higher LBM in tibolone users compared with nonusers, the latter supported by several studies reporting positive effects of tibolone on total LBM (e.g., Refs. 20, 34, 56).

The sisters using estrogen-containing preparations had significantly smaller thigh fat area, subcutaneous fat area, and relative proportion of fat in the whole thigh area, as well as within the muscle compartment, than their cotwins with no HRT history. In addition, they also had lower percent body fat compared with their sisters. A previous study reported that abdominal fat percentage increased with placebo compared with a combined estrogen and progestogen treatment, although no difference in total body fat was observed (18). Moreover, in another study the placebo group gained more weight over a 5-yr period than women taking an estrogen-containing preparation, a result due in particular to changes in body fat (23). Similar results have been obtained elsewhere as well (29), although reports documenting contradictory results (2) or no effects (27, 51) of HRT on body fat have also been published. The assumption that HRT could, however, prevent the accumulation of excess body fat may hold clinical relevance, since the amount of adipose tissue, especially within the fascia surrounding skeletal muscle, has been documented to be related to insulin resistance (1) and, at the level of the whole body, adiposity has further been strongly associated with adverse health outcomes such as cardiovascular disease, diabetes, and cancer (10). The findings of our study suggest that both estrogen-related and tibolone therapies exert positive effects on muscle composition, irrespective of the target tissue.

The precise molecular level mechanisms responsible for the changes found in muscle phenotypes due to HRT use remain to be clarified. This issue is further complicated by the diversity of HRT regimens. Data on human studies demonstrate that both known estrogen receptors (ERs), ERα and ERβ, are expressed in skeletal muscle (33, 62a, 63). Intriguingly, in our previous study (40) with postmenopausal women a menu of changes in the expression levels of specific genes in muscle samples was observed after 1 yr without HRT during the early postmenopausal years, whereas HRT use was shown largely to counteract these changes. In light of these data, skeletal muscle can be considered potentially to be responsive to estradiol signaling. Furthermore, experimental animal studies by D’Eon and colleagues (11) support a role for estrogen itself in reducing overall adiposity by downregulating the expression of lipogenic genes not only in the adipocytes but also in skeletal muscle. Moreover, an alternative HRT, tibolone, along with estradiol has been reported effectively to restore the episodic release of growth hormone (12), which, again, has been suggested to promote lipolysis, resulting in a potential anabolic effect on muscle tissue and a decrement in the amount of fat in the muscle compartment (22, 55). Consequently, estradiol should be recognized when the intricate pathways leading to muscle weakness are constructed.

Our cotwin analysis, in which medication-exposed MZ cotwins are compared with their unexposed sisters, holds several advantages over traditional case-control and experimental designs. Cotwin analysis intrinsically adjusts for genetic factors and in addition for a number of shared environmental factors starting from childhood. Therefore, our data set together with the analytical strategy offer a powerful tool to evaluate the association between long-term HRT and skeletal muscle characteristics. Our analyses are based on a relatively small number of identical female twin pairs. This number of participants in a study design with discordant MZ twins, however, is comparable to the number of participants in previous reports (e.g., Refs. 8, 32, 39) and has sufficient power to detect clinically relevant differences. We are, however, well aware that in the subgroup analysis the number of pairs using tibolone is small and therefore the analysis statistically underpowered. It should be noted that when using Wilcoxon’s signed rank test for sample sizes smaller than $n = 5$ it is impossible to receive $P$ values at or beyond 0.05. With our data
the CIs, however, clearly indicate the significance of the main results.

This genetically controlled case-control study showed that long-term HRT was associated with better mobility, greater muscle power, and favorable body and muscle composition among 54–62-year-old women. The results indicate that HRT is a potential agent in preventing muscle weakness and mobility limitation in older women.

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